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DEPARTMENT OF MICROBIOLOGY

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Dear Dr. Lederberg,

I would like to take this opportunity to apologize for the delay in thanking you for the cultures which you so generously supplied me last month. The cultures are indeed the types of mutants I had desired. However, in my initial characterization of these cultures, I was prompted to refrain from writing you because of some observations I made. At this time I think that I have adequately surveyed these particular features and I would like to ask your advice regarding them.

In our standard departmental broth, the fermentations, which I am most interested in, showed their traits of non-fermentation of the indicated sugars within 24 hours with the exception of W-1 which fermented maltose. In 3 to 5 days, all the sugars were fermented. (This medium consists of 1% sugar, 1% Proteose Peptone #3 and Brom cresol purple as indicator.) Realizing that perhaps the medium might be at fault, I prepared EMB using your formula of EMB synthetic medium with the addition of 0.3% beef extract and 1% Peptone. I also prepared another solid medium consisting of 1% Peptone, 0.3% beef extract, 1% sugar, 1.5% agar and Brom thymol blue as indicator. In both these media the base was autoclaved and the concentrated sugars, sterilized by filtration, were added to the base prior to use.

On these solid media, the cultures were true as you had indicated in the first 72 hours. A broth was then prepared using 1% Peptone, Brom thymol blue as indicator and 1% sugar contending that perhaps the Proteose Peptone #3 was enabling the organisms to somehow synthesize the necessary fermentation enzymes. Again, the cultures fermented all the sugars within 72 hours with the possible exception of lactose and xylose in the case of W-1177. However, these cultures in 5 days are weakly acid and the solid Brom thymol blue and EMB media after 5 days are also tending to become acid probably indicating the fermentation of the sugars. As a further test before drawing any conclusions, I transferred the broth cultures of the mutants as soon as they produced acid and gas to fresh media of the same kind and in 18 hours observed acid and gas formation.

These observations force me to believe that the sugar fermentations are not true non-fermentation mutants but examples of adaptation, the case of W-1177 being slower than the rest, particularly with lactose and xylose.

The problem I wrote you about in my previous letter necessitates that during the course of rabbit immunization, my cultures must remain true. I intend to study the antibodies formed to see if the mutants are antigenically

similar or dissimilar and also to use the antibodies in various ways to determine if I can induce forward or reverse mutations.

As I stated previously, I would like to work with a parent strain and 2 - 3 mutants which are 1, 2 and 3 traits (or more) removed from the parent. The cultures you sent, K-12, Y-53, W-1, W-1177 and W-1 are exactly the types I desire because of their sugar fermentation characteristics. Their untoward behavior, however, forces me to hesitate to use them.

I would therefore appreciate any advice you could give me. Perhaps you could suggest some other cultures in your collection which would be more advantageous for my study.

Sincerely yours,

Carl J. Wust

CJW:bmr

enc.